ferric–ferrous electrode potential is calculated to be 0.782 ± 0.001 volt at 25°.

Although our values for K and the ferric–ferrous electrode potential are considerably higher than the commonly accepted values for these constants, there is evidence other than that presented here to indicate that the higher values are correct. Thus, Bray and Hershey[9] developed a method for calculating the amount of hydrolysis in a solution of ferric ion, and with the aid of this hydrolysis correction they have recalculated the data of Noyes and Brann[1] and concluded that K should have the value 0.363 instead of 0.128. Similarly they concluded that the value of Popoff and Kunz[2] for the molal ferric–ferrous electrode potential should be raised from 0.748 to 0.772 volt. Their recalculated values for these constants thus agree more closely with our values than with the previously accepted ones.

The method of Bray and Hershey[9] for hydrolysis correction was applied to the data obtained in this investigation. The correction, however, was appreciable only for the last point on the curve in both series; furthermore, application of the correction would have changed the slope in this region of the curves upward in an unaccountable manner. Consequently such an hydrolysis correction was omitted.

(9) Bray and Hershey, THIS JOURNAL, **56**, 1889 (1934).

The suggestions and assistance of Professor George Scatchard of this Institute in the interpretation of our data are gratefully acknowledged.

Summary

The equilibrium constant of the reaction $Fe^{+++} + Ag \rightleftarrows Fe^{++} + Ag^{+}$ has been redetermined with perchlorate as the acid constituent. Two series of experiments have been conducted at 25° with a different constant ratio of perchloric acid to ferric perchlorate in each series. The data have been plotted as $\log K + 2.02\,\mu^{1/2}$ against μ and extrapolated to zero ionic strength. The mean value thus determined for K is 0.531. From this value of K the molal ferric–ferrous electrode potential has been calculated as 0.782 volt.

CAMBRIDGE, MASS. RECEIVED MARCH 15, 1935

[CONTRIBUTION FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

The Ultraviolet Absorption Spectrum of Pepsin

BY GEORGE I. LAVIN AND JOHN N. NORTHROP

Introduction

Recent investigations of Lavin, Northrop and Taylor[1] and of Gates[2] have shown that pepsin has an absorption band in the ultraviolet at 2500–3000 Å. This band is typical of the proteins and has been attributed to the absorption of the aromatic amino acids, tyrosine and tryptophan. Thus Stenström and Reinhard[3] found that gelatin, which contains very little tyrosine and no tryptophan, did not show this absorption band.

A large amount of work has been done on the absorption spectra of the amino acids and most of the results indicate that only the aromatic acids have selective or band absorption in the region 2500–3000 Å. Where the investigators have determined the extinction coefficients, using the usual photometers and line sources, they have, in general, found only one wide band. However, if a continuous light source is used, it is possible to show that tyrosine, tryptophan and phenylalanine have narrow absorption bands as well as the well-known broad band.

Among others, Ross[4] has found that tyrosine has two absorption bands, one at 2840 Å. and another at 2760 Å. In the case of tyrosine, Stenström and Reinhard have shown that the position of the bands is dependent on the hydrogen ion content of the solvent. Ross also found that tryptophan has a narrow band at 2900 Å. and a broad region of absorption from 2850 to 2600 Å. The absorption of phenylalanine was found to consist of five narrow bands at 2680, 2640, 2585, 2525 and 2480 Å.

Since our work on the absorption spectrum of pepsin had shown the presence of narrow bands, an attempt has been made to explain the absorption in this region in terms of the individual aromatic amino acids.

(1) Lavin, Northrop and Taylor, THIS JOURNAL, **55**, 3497 (1933).
(2) Gates, *J. Gen. Physiol.*, **17**, 797 (1933–34).
(3) Stenström and Reinhard, *J. Biol. Chem.*, **66**, 819 (1925).
(4) Ross, *ibid.*, **104**, 531 (1934).

Results

The bands are rather faint and it is very difficult to obtain them by means of the usual procedure for determining extinction coefficients—especially if a line source is used. In the present work a continuous light source (a hydrogen discharge tube) was used. Kistiakowsky and Arnold[5] have shown that low temperature sharpens the absorption bands of aromatic organic compounds and this fact has been used to advantage. The apparatus that we have employed has been described in a previous communication.[1] The spectrograph used was the small one manufactured by Hilger. Northrop's crystalline pepsin was used in all the experiments.

One of the difficulties in the low temperature spectroscopy of proteins is to find a suitable solvent. This solvent must neither inactivate nor denature the material; it must not have an absorption band in the region of absorption of the substance and it must not become opaque at the low temperatures. Glycerol is the one solvent, found so far, which has most of these properties. However, it was found that if the solution were cooled below $-100°$ the glycerol cracked. For this reason it was not possible to go much below this temperature.

The results obtained thus far show that pepsin has a number of narrow absorption bands in the region 2500–3000 Å. These are represented in the accompanying figure. We have repeated the work on the absorption spectrum of tyrosine, tryptophan and phenylalanine and these are also shown in the same figure.

Our interpretation of the narrow bands in the absorption spectrum of pepsin, based on the band absorption of the amino acids, is to attribute the band at 2900 Å. to tryptophan, the one at 2850 Å. to tyrosine and those at 2500–2700 Å. to phenylalanine.

The tryptophan and tyrosine bands in pepsin can be obtained even at room temperature and, as might be expected, the narrow tyrosine band is weakened since it falls in the region of continuous absorption of the tryptophan. This is also true of certain of the phenylalanine bands. The phenylalanine bands can be seen at room temperature but it is necessary to cool the solution if even approximate measurements are to be made.

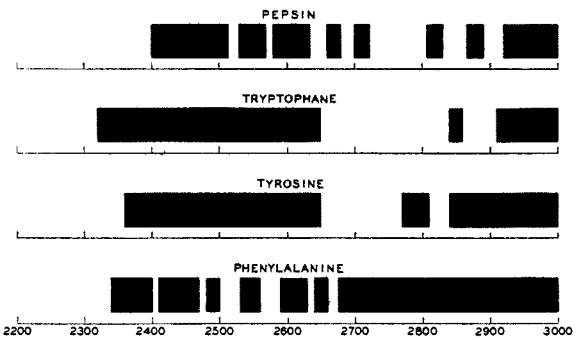

Fig. 1.

Lowering the temperature sharpens all the bands. Since in certain cases the edges of the bands are not as sharp as might be desired the positions may have to be changed slightly when equipment for more precise measurement is available. The work is being continued on pepsin and other proteins.

Summary

It has been shown that pepsin has a number of narrow absorption bands in the region 2500–3000 Å.

An attempt has been made to explain these bands in terms of the absorption of individual aromatic amino acids in the molecule.

(5) Kistiakowsky and Arnold, THIS JOURNAL, **54**, 1713 (1932).

PRINCETON, N. J. RECEIVED MARCH 20, 1935